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(71) Applicants *(for all designated States except US)*: UNIVERSITY COLLEGE LONDON [GB/GB]; Rowland Hill Street, London NW3 2PF (GB). NATIONAL INSTITUTE OF BIOLOGICAL STANDARDS AND CONTROL [GB/GB]; Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CHARALAMBOUS, Bambos, Michael [GB/GB]; 45 The Baulk, Biggleswade, Bedfordshire SG18 0PX (GB). FEAVERS, Ian, Michael [GB/GB]; 3 Ceasars Road, Wheathamstead, Hertfordshire AL4 8NR (GB).

(74) Agents: DANIELS, Jeffrey, Nicholas et al.; Page White & Farrer, 54 Doughty Street, London WC1N 2LS (GB).

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(57) Abstract

A component for a vaccine against serogroup B meningococci, which comprises a mimotope of a surface lipooligosaccharide of a serogroup B meningococcus.

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COMPONENT FOR VACCINE

Field of the Invention

The present invention relates to a component for a vaccine against meningococci and to a vaccine comprising such a component.

Background to the Invention

Meningococcal meningitis is a serious illness having a mortality rate of approximately 10%. Survivors of meningococcal meningitis may be left with various degrees of permanent neurological damage. In England and Wales alone there are around 1300 to 1500 meningococcal infection cases reported each year. Meningococci are the bacteria responsible for the disease and are divided into a number of different serogroups according to their immunological activity. Of these, serogroups A, B and C are responsible for about 90% meningococcal cases worldwide, whilst serogroup B accounts for more than 50% of the cases in the UK and many other European countries.

Research into potential vaccines against meningococci has lead to some success. The meningococci each have polysaccharide-containing coats and it has been found in serogroups A and C that such polysaccharides chemically conjugated to T-dependent protein antigens give rise to promising immunogenicity data from clinical trials in infants. However, only a poor immune response is elicited against the corresponding B polysaccharide, which is thought chemically to resemble human cell surface carbohydrates and may therefore be tolerated by the human immune system. As part of the polysaccharide coat, meningococci contain a lipooligosaccharide (LOS) component which is a surface glycolipid that forms a major outer membrane component and possesses a terminal galactose acceptor site for sialic acid.

It is thought that sialylation of the LOS in immunotype B meningococci enhances the ability of the organism to evade the human immune response. Accordingly, researchers are investigating alternative target antigens on the surface of serogroup B meningococci, especially various protein antigens more likely to elicit an immune response in humans.

Summary of the Invention

The present invention aims to overcome the disadvantages of the prior art and provide a component for a vaccine against serogroup B meningococci.

The present invention provides a component for a vaccine against serogroup B meningococci, which comprises a mimotope of a surface lipooligosaccharide of a serogroup B meningococcus.

It has been surprisingly found that a mimotope (conformational homologue) of LOS is capable of eliciting an immune response *in vivo*. The mimotope is preferable antigenically cross-reactive with a monoclonal antibody of high affinity to the surface lipooligosaccharide. Typically, by high affinity is meant having an affinity constant of 10^5M^{-1}, preferably at least 10^6M^{-1}. Such monoclonal antibodies include the following:

Immunotype	Antibody Name	Author
LOS 1	3G3-1-8C	Zollinger, W
LOS 2	F1-5H 5/ID9	Sacchi, C
LOS 3,7,9	4A8-B2	Poolman, J
LOS 3,7,9	9-2-L397	Zollinger, W
LOS 8	6E7-10	Zollinger, W
LOS 10	5B4-F9-B10	Zollinger, W

These monoclonal antibodies may be obtained from the National Institute of Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK and may also be obtained through ECACC.

Monoclonal antibodies of high affinity to LOS may be prepared using outer membrane complexes as immunizing and detecting antigens according to established protocols (6) (7).

The mimotope preferably comprises a peptide epitope which may be identified by screening a peptide library with the monoclonal antibody. Typically, a peptide library such as a heptapeptide library preferably containing all possible amino acid sequences should be used to give the greatest diversity of potential epitopes against which antigenic cross-reactivity with the monoclonal antibody can be assessed. Typically, a random peptide library of this nature is used.

In the present invention it was found that a heptapeptide library was conveniently challenged with the relevant monoclonal antibody and the following peptide sequences were found to be particularly useful:

IHRQGIH HIGQRHI LPARTEG GETRAPL APARQLP PLQRAPA
KQAPVHH HHVPAQK LQAPVHH HHVPAQL LPSIQLP PLQISPL
NELPHKL LKHPLEN KSPSMTL LTMSPSK AGPLMLL LLMLPGA
WSPILLD DLLIPSW LSMHPQN NQPHMSL HSMHPQN NQPHMSH
SMYGSYN NYSGYMS TNHSLYH HYLSHNT HAIYPRH HRPYIAH
TTYSRFP PFRSYTT TDSLRLL LLRLSDT SFATNIP PINTAFS

Preferably, the heptapeptide sequence is SMYGSYN or APARQLP.

According to the present invention, the peptide epitope may comprise a subsequence of any one of the heptapeptide sequences

or may be present in a longer peptide incorporating any one of the above heptapeptides or sub-sequences therefrom.

In a further embodiment, the mimotope may comprise an oligopeptide which is structurally more constrained than a linear form of the oligopeptide. An unsubstituted linear oligopeptide such as present free in solution would normally be able to assume a large number of different conformations. In contrast, an oligopeptide which is structurally constrained, perhaps by having one or usually two or more substituents which reduce in number the possible conformations which it can assume, is preferred as the mimotope of the present invention. It is thought that peptides which assume fewer conformations or which have their conformations locked are more likely to elicit an immune response because they present to the binding portion of antibodies a structurally constrained epitope.

Substituents such as covalent linkages to further peptide chains or intramolecular linkages will structurally constrain the oligopeptide. For example, the oligopeptide may form part of the primary structure of a larger polypeptide containing the amino acid sequence of the oligopeptide. Preferably, the oligopeptide comprises a cyclic peptide, as discussed in further detail below.

Other substituents include covalent linkages to other moieties such as macromolecular structures including biological and non-biological structures. Examples of biological structures include carrier proteins such as those described below for enhancing the immunogenicity of the mimotope. Examples of non-biological structures include lipid vesicles such as micelles and the like.

In a preferred embodiment, the oligopeptide comprises a cyclic peptide. Use of a cyclic peptide is thought to be superior to a linear peptide because the cyclic peptide is able to assume fewer conformations that the linear peptide and is therefore structurally more constrained. Typically, the cyclic peptide comprises a cyclised portion, which cyclised portion preferably comprises an amino acid sequence, the terminal amino acids of which are linked together by a covalent bond. The covalent bond is conveniently a disulphide bridge, such as found between cysteine residues. The cyclised portion typically comprises a heptapeptide and this heptapeptide can conveniently form part of the amino acid sequence which is flanked by the amino acids which are linked by the covalent bond to form the cyclised portion. The N terminal of the cyclic peptide preferably comprises an A residue.

The oligopeptide may be generated from a peptide library in the same way as described above. A typical oligopeptide is shown in Figure 6 of the drawings.

It is preferred that the heptapeptide of the cyclised portion has the sequence SWXHXPY, wherein each X is the same or different and is an amino acid, preferably a naturally-occurring amino acid. Particularly useful sequences are SWLHMPY, SWMHMPY, SWDHMPY and NTIGGYE. SWLHQPY is a particularly preferred heptapeptide sequence which may be flanked by cysteine residues and is preferably terminated at the N terminal by an A residue. It is preferred that the N terminal A residue is unacetylated and preferably does not carry a charge as this may interfere with the immunogenicity of the oligopeptide.

It is thought that at least a part of the peptide epitope is present in the heptapeptide.

In addition to the above linear and cyclic peptides, it is possible to use, instead of peptides based on naturally-occurring L-amino acids, peptides comprising corresponding D-amino acids with the sequence of amino acids running in reverse order. These D-amino acid based peptides are sometimes termed retropeptides and can be more immunogenic and less prone to proteolysis than their L-amino acid counterparts.

In a preferred embodiment, a carrier is conjugated to the mimotope to enhance the immunogenicity thereof. A number of carriers are known for this purpose, including various immunogenic protein-based carriers such as diptheria toxoid (dt CRM_{197}) or tetanus toxoid and T-cell peptide.

The component for the vaccine may incorporate a plurality of mimotopes of the surface lipooligosaccharide in which each mimotope is the same or different. By having more than one mimotope, the immunogenicity of the component may be enhanced. The plurality of mimotopes may be conjugated together, for example using a polylysine to which each mimotope is conjugated.

Preferably, the serogroup B meningococcus is *Neisseria meningitidis*.

In a further aspect, the present invention provides a vaccine comprising a component according to any one of the preceding claims optionally together with an adjuvant so as to increase the immune response thereto.

In a further aspect, the present invention provides a method for producing a component for a vaccine against serogroup B meningococci, which method comprises screening with a monoclonal antibody a mimotope library comprising candidate mimotopes, and selecting at least one candidate mimotope with high affinity to the monoclonal antibody, wherein the monoclonal antibody has high affinity to a surface lipooligosaccharide of a sergroup B meningococcus.

The monoclonal antibody is preferably as described above and the mimotope preferably comprises a peptide epitope as discussed above. The mimotope library preferably comprises a peptide library comprising candidate peptides, such as those described above. Conveniently, the peptide library comprises a peptide phage-display library.

Brief Description of the Drawings

The present invention will now be described in further detail, by way of example only, with reference to the accompanying drawings, in which:

FIGURE 1 shows a graph of real time interaction kinetics of a monoclonal antibody with a peptide vaccine component according to the present invention;
FIGURE 2 shows a graph of real time binding interaction of the monoclonal antibody with immobilised LOS protein of *Neiseria meningitidis;*
FIGURE 3 shows an immunological response of mice sera to LOS 3, 7, 9;
FIGURE 4 shows a further immunological response of mice sera to LOS 3, 7, 9;
FIGURE 5 shows the immunological responses of individual mice sera against LOS 3, 7, 9;

FIGURE 6 shows a schematic representation of a cyclic heptapeptide at the free N-terminus of the M13 phage membrane protein PIII;
FIGURE 7 shows initial rate constant for binding of peptides of the present invention to monoclonal antibody 9-2-L379;
FIGURE 8 shows a bar graph of phage particles recovered after each round of bio-panning with the monoclonal antibody; and
FIGURE 9 shows a bar graph of phage particles bound in phage binding assays using various cyclic heptapeptides.

Detailed Description of the Invention

Example 1

Introduction

Serogroup B meningococcus immunotype LOS3,7,9 is the immunotope responsible for >50% of cases of bacterial meningitis seen in Europe. An aim of this study is to determine whether linear peptides, selected by binding to LOS3,7,9 immunotyping antibodies, will elicit protective antibodies that cross-react with LOS3,7,9, when used to immunise animals. Initially, the binding characteristics of two LOS3,7,9 immunotyping antibodies were studied by resonant mirror analysis. The antibody with the greatest affinity for the LOS antigen was then chosen to "biopan" a heptapeptide phage-display library.

Evidence is presented that such a selected peptide, when used to immunise mice, generates a cross-reactive immune response against *Nm*LOS3,7,9.

METHODS

Monoclonal immunotyping antibodies : The two immunotyping monoclonal antibodies to be studied, 9-2-L379 and 4A8-B2, (kind gifts from Drs. Zollinger and Poolman, respectively) were

purified from ascitic fluid by standard Protein G affinity chromatography, and made up to a stock concentration of 1 μM.

Heptapeptide phage display library : An M13 peptide phage display library (Ph.D.7) from New England Biolabs was used. The DNA sequences encoding random heptapeptides were inserted in to gene III, which encodes a surface protein expressed at one tip of the phage. The 21 nucleotide insertions were 3' to the sequence coding for the leader sequence so that the peptide is free at its N-terminus and attached to Protein III via its C-terminus.

Immobilisation of *Neisseria meningitidis (Nm)*LOS3,7,9 to the biosensor cuvette surface : To determine the monoclonal antibody binding kinetics, purified LOS was prepared from isolate K454 (B:15:P1.7,16 : L379) as described previously (2). LOS was then biotinylated according to Romero & Outschoorn (3), and immobilized to a biotin-coated biosensor cuvette surface via a streptavidin bridge according to IAsys, Plc. A final BSA blocking step was performed to reduce non-specific binding. The LOS coated biosensor surface was treated with 20mM HCl to remove any weakly bound substances before interaction kinetics were performed, and also to regenerate the LOS surface after each antibody interaction. The kinetic data for both the antibodies were all determined from the same LOS-coated biosensor cuvette.

Immobilisation of the consensus peptide to the biosensor cuvette surface : The peptide was biotinylated by Chiron Technologies, Ltd to permit immobilisation to a biotin-coated biosensor cuvette via a streptavidin bridge.

Resonant mirror biosensor analysis : The binding kinetics of the monoclonal antibodies were determined to the immobilized *Nm*LOS3,7,9 with an IAsys resonant mirror biosensor (Affinity Sensors, Saxon Way, Bar Hill, Cambridge). Real-time kinetic analyses were performed in PBS / 0.05 % (v/v) Tween-20 at 25°C, according to the methods described by the manufacturer. The kinetic data were analysed by curve fitting software (FASTfit v2.01, Affinity Sensors), and the interaction profiles were overlaid with the FASTplot™ software (Affinity Sensors). Dissociation (K_{OFF}) rates were determined by dilution to zero concentration of unbound antibody in the biosensor cuvette at high concentrations of interacting antibody. K_{OFF} and K_{Ass} rates were calculated from the arc second response per second over various periods of time, and averaged. K_{Ass} rates were plotted as a function of antibody concentration, and the slope of the linear regression gave the K_{ON} rate. The Affinity constant (K_A) was calculated for each antibody from K_{ON} / K_{OFF} and the Dissociation equilibrium constant (K_D) from K_{OFF} / K_{ON}. An approximate K_D was also obtained by curve fitting of the total extent of antibody binding versus the antibody concentration. In addition approximate K_{OFF} values were obtained from the Y-intercept of the linear plot of K_{ON} versus antibody concentration.

Similarly, the binding kinetics of 9-2-L379 used to biopan the heptapeptide library were determined to the immobilised consensus peptide. The concentration of Tween-20 was 0.5% (v/v) in the PBS, identical to the biopanning buffer.

Biopanning a heptamer peptide phage-display library : The immunotyping monoclonal antibody 9-2-L379 was immobilised to a carboxymethylated dextran coated biosensor cuvette surface by

succinimide ester chemistry according to the manufacturers instructions (Affinity Sensors). To enrich for peptides that react with this antibody 2 x 10^{11} M13 phage particles expressing random heptapeptides on their surface were allowed to react with the immobilised antibody for 60 min at 25°C in PBS buffer containing 0.50 % (v/v) Tween-20. A high Tween concentration was used to reduce non-specific phage binding to the surface. The cuvette surface was washed 6 times with the same PBS / 0.5 % Tween buffer. The remaining bound phage were eluted from the cuvette surface by addition of Tris-glycine buffer, pH 2.2. After 10 min the buffer was carefully pipetted into a sterile Eppendorf tube, and neutralised with 1 M Tris. The harvested phage particles were amplified by infection of *Escherichia coli* and titrated. 2 x 10^{11} phage particles from this 1st biopan were then reacted with the immobilised antibody as before. The phage from the second biopanning were then amplified and titrated, and 2 x 10^{11} phage particles biopanned for a third time.

Phage from all three biopannings were isolated, and the region of the DNA insertion expressing the heptapeptide was sequenced.

Peptide synthesis: The consensus peptide sequences were identified, and one peptide (#12) was synthesised with a cysteine bridge in the C-terminal position, the same site as its attachment to the M13 phage Protein III (Chiron Technologies, Ltd). This enabled attachment to a sulfhydryl-coated ELISA plate for immunoassays, or the conjugation to the carrier protein, diptheria toxoid CRM_{197} for immunising animals, or to biotin for immobilisation to the resonant mirror biosensor cuvette surface.

Immunisation of mice: 2 independent groups of 10 BALB/c mice were immunised subcutaneously (and boosted at 4 weeks) with : 20µg of the consensus peptide #12; the peptide conjugated to CRM_{197} carrier; the whole M13 phage displaying the consensus peptide; suitable buffer +/- CRM_{197} as controls. Immunisations were performed with or without adjuvant. When adjuvant was included Freund's complete adjuvant (CFA) was used in the initial immunisation, and Freund's incomplete adjuvant in the booster. Serum samples were obtained at 4 weeks by exsanguinating 5 out of the 10 mice immunised; the remaining 5 mice were boosted and exsanguinated at 6 weeks.

Antibody assays: Cross-reactive anti-*Nm*LOS3,7,9 antibody activity in pooled (5 mice) or individual serum samples was determined by a solid phase immunoassay with microtiter plates (Nunc, Roskilde, Denmark) coated with purified *Nm*LOS 3,7,9 (2) overnight at 37°C, according to Verheul,. *et al* (4). The secondary antibody (diluted 1 : 2000) was a rabbit anti-mouse IgG conjugated to peroxidase. The magnitude of the cross-reactive serum antibody response to LOS3,7,9 was calculated from the geometric means obtained from double $\log_{10}$ plots of OD_{450} and the reciprocal of the serum dilution. The ratio of the anti-$\log_{10}$ reciprocal serum dilution between the immunised and the control mice at -1.0 $\log_{10}$ OD_{450} gave the magnitude of the response.

Competition experiments: Various concentrations of the unconjugated consensus peptide #12 were incubated with 150nM of 9-2-L379 antibody for 3 hours at room temperature prior to interaction with the immobilized *Nm*LOS3,7,9. To control for non-specific interaction between the peptide and the immobilised LOS, the binding interaction profiles of peptide #12 were

subtracted from the interaction profiles observed with peptide #12 preincubated 9-2-L379. The subtraction was performed using the Fastplot™ software (Affinity Sensors).

Results

Binding kinetics of the immunotyping monoclonal antibodies: The affinity constants for 4A8-B2 and 9-2-L379 were 4.0 x 10^6 M^{-1} and 1.3 x 10^8 M^{-1}; the latter antibody having a 44 - fold faster K_{ON} rate (5). Since 9-2-L379 had a greater affinity towards the LOS3,7,9, we chose it to undertake the biopanning of the random heptapeptide library.

Biopanning experiments: Data from the biopanning experiments revealed the presence of several consensus peptide sequences (see Table I). This initial study focused on consensus peptide #12 S~M~Y~G~S~Y~N.

Consensus peptide binding kinetics: Fig. 1 shows the binding interaction of 9-2-L379 with immobilised consensus peptide #12. The consensus peptide #12 conjugated to biotin was immobilised to a biotin-coated biosensor cuvette via a streptavidin bridge. Various concentrations of the monoclonal antibody 9-2-L379 were reacted with the immobilised peptide in PBS / 0.5% (v/v) Tween-20 at 25°C. To determine the dissociation (K_{OFF}) rate the antibody was diluted to zero dilution after the antibody had interacted with the immobilised peptide. The error bars in Fig. 1 are S.D. Real-time binding kinetics performed by resonant mirror analysis revealed that the 9-2-L379 antibody bound to the consensus peptide #12 with a K_{ON} rate of **3.1(±0.3) x 10^4 M^{-1} s^{-1}** and a K_{OFF} rate of **0.013(±0.0003)** (Fig. 1). This gives an affinity constant (K_A) for 9-2-L379 binding to peptide #12 of **2.6**

(±0.3) x 10^6 M^{-1}, compared to a K_A of 1.3 x 10^8 M^{-1} to its LOS3,7,9 idiotype.

Competition assay: Fig. 2 shows in real-time the binding interaction of antibody 9-2-L379 with immobilised *Nm*LOS3,7,9. Antibody 9-2-L379 (130nM) was preincubated with either 50nM ( ), 25nM (-) of the consensus peptide #12, or buffer (-) for 3h at room temperature prior to interacting with LOS3,7,9 immobilised on the biosensor cuvette surface. The binding interaction profiles of 50nM and 25nM peptide #12 were subtracted from the respective 9-2-L379 + peptide binding profiles. The consensus peptide #12 competed with the binding interaction of the 9-2-L379 antibody to the immobilised *Nm*LOS3,7,9 (Fig. 2). There was also a slow interaction of the peptide with the immobilised LOS that appeared to be aggregation on the LOS surface, and was subtracted from the antibody + peptide interaction profiles.

Immunological data: We studied a variety of immunisation procedures in BALB/c mice, but the only significant cross-reactive response to *Nm*LOS3,7,9 was generated from mice immunised with peptide #12 conjugated to CRM_{197} administered in Freund's adjuvant (Figs. 3 and 4). According to Fig. 3, mice were immunised on day 0 and day 28, and the immunoreactivity of pooled sera from day 28 and day 42 were assayed against *Nm*LOS 3,7,9 (left panel). To calculate the magnitude of the immunological response the geometric mean was calculated from a double log_{10} plot (right panel). Key : PBS + Adj = phosphate buffered saline + Freund'adjuvant; CRM + Adj = CRM_{197} + Freund'sadjuvant; pep12-DT+Adj = peptide #12 conjugated to dt CRM_{197} + Freund's adjuvant; M13-pep12 = M13 phage expressing the peptide #12 on the surface. Having determined the geometric mean, we calculated from this the reciprocal serum dilution

required to give the same OD_{450} reading for the peptide, and control (buffer + adjuvant) pooled sera. The ratio at an OD_{450} - 1.0 log_{10} was 2630 : 603 = 4.4. Mice immunised with diptheria toxoid (CRM_{197}) + adjuvant generated a small, but insignificant cross-reactive response to *Nm*LOS3,7,9. However, the geometric mean was not parallel to either the peptide immunised or the control sera (Fig 3, right panel). Fig. 4 shows the averaged immunological data of pooled sera from 2 independent experiments. Mice were immunised with either PBS + adjuvant (control), or with 20µg peptide #12 - DT + adjuvant. The data were linearised by a double log_{10} plot and the geometric mean was determined. Error bars indicate the range of the average values.

Fig. 5 shows the immunological responses of individual mouse sera from two independent experiments that have been immunised with either PBS (control; n = 7), or peptide #12 conjugated to Diptheria Toxoid, both in the presence of Freund's adjuvant. Mice were exsanguinated after 6 weeks, following a booster injection at 4 weeks. The bar represents the mean of each group. The Figure shows individual sera diluted 1 : 100 from control and peptide #12-diptheria toxoid + adjuvant immunised groups mice. The antibody responses to immunisation with the peptide #12-DT + adjuvant of all the animals tested were consistent, and the difference between their reactivity by solid phase immunoassay was highly significant (**$p < 0.0001$**) : **0.19 ± 0.01** (Mean ± SEM; n = 7) control; **0.35 ± 0.02** (Mean ± SEM; n = 10). Furthermore, the immunological cross-reactivity was increased on boosting at 4 weeks by the same percentage (24%) as the reactivity increased towards the consensus peptide #12 (data not shown).

Conclusion

Several consensus peptides were isolated by biopanning with a monoclonal antibody to *Nm*LOS3,7,9. This antibody had an affinity constant to peptide #12 (S~M~Y~G~S~Y~N) of 2.6×10^6 M^{-1}, which is comparable to the binding of the weaker binding immunotyping antibody 4A8-B2 (4.0×10^6 M^{-1}). When peptide #12 conjugated to Diptheria Toxoid in the presence of Freund's adjuvant was used as the immunogen it elicited a significant cross-reactive polyclonal antibody response to *Nm*LOS3,7,9. This response was consistent in all the animals tested, persisted over the 6 week period of the investigation, and was boosted by 24%.

Table I. Heptapeptides displayed on M13 phage after 3 rounds of biopanning against the anti-*Nm*LOS3,7,9 monoclonal antibody 9-2-L379.

Frequency	Peptide sequence
1	~I~H~R~Q~G~I~H
3	~L~P~A~R~T~E~G
3†	A~P~A~R~Q~L~P~
1	K~Q~A~P~V~H~H~
1	L~Q~A~P~V~H~H~
3	L~P~S~I~Q~L~P~
3	N~E~L~P~H~K~L~
2	K~S~P~S~M~T~L~
2	A~G~P~L~M~L~L~
1	~W~S~P~I~L~L~D~
2	L~S~M~H~P~Q~N~
1	H~S~M~H~P~Q~N~
3*	S~M~Y~G~S~Y~N~
1	~T~N~H~S~L~Y~H
1	H~A~I~Y~P~R~H~
1	T~D~S~L~R~L~L~
2	T~T~Y~S~R~F~P~
1	S~F~A~T~N~I~P~

† To be synthesised and tested in mice

* Consensus peptide #12 used in this study

Example 2

Introduction

Following on from the work on linear peptides in Example 1, this Example investigates the potential of cyclic peptides to act as structural mimics (mimotopes) of meningococcal LOS. A phage library displaying random cyclised heptapeptides that are structurally constrained by a disulphide bridge between two cysteine residues as shown in Figure 6 was subjected to biopanning to establish whether such peptides are better mimotopes than linear peptides.

Methods and Results

Biopanning a cyclic peptide phage-display library: An M13 bacteriophage library displaying random cyclic heptapeptides at the free N-terminus of the PIII membrane protein (PhD C7C, Biolabs) was bio-panned against the anti-*Nm*LOS mAb 9-2-L379 coated onto microtitre wells. M13 plaques were picked from an *E. coli* lawn after four rounds of enrichment with the anti-*Nm*LOS 3,7,9 mAb 9-2-L379. DNA was prepared by standard procedures and sequenced from primers provided in the phage library kit (Ph.D-C7C, New England Biolabs). At each round of bio-panning 10^{11} phage were interacted with the immobilised mAb. Non-specific phage were removed by six rinses in neutral phosphate buffered saline with 0.5% Tween-20. The interacting phage were removed by addition of pH2.2 Tris-glycine buffer. After neutralisation with Tris pH8.8 the phage were titred and then amplified by transfection of *E. coli* (ER 3257; supplied by New England Biolabs). mAb 9-2-L379 at 50µg/ml was used to coat the microtitre wells in the first three rounds. In the fourth round 10^{11} phage were interacted with decreasing concentrations of mAb, as indicated. The peptide sequences are expressed at the N-terminus

of the PIII protein of the M13 phage. The more prevalent sequence (C10) obtained after four rounds of enrichment is shown schematically in Figure 6. The residues SWLHQPY are randomly displayed by the phage whereas the others are non-variable.The line between the cysteine residues represents a covalent linkage via a disulphide bridge.

Table II shows the peptide sequences of enriched phae clones.

Phage number	Peptide sequence	Frequency 50µg/ml 9-2-L379	Frequency 5µg/ml 9-2-L379	Frequency 0.5µg/ml 9-2-L379
C10	NH_2-AC**SWLHQPY**CGGGS...	13/33 (39%)	13/32 (41%)	18/32 (56%)
B5	NH_2-AC**SWLHMPY**CGGGS...	2/33 (6%)	4/32 (13%)	0/33
C19	NH_2-AC**SWMHMPY**CGGGS...	5/33 (15%)	2/32 (6%)	4/32 (13%)
B11	NH_2-AC**SWDHMPY**CGGGS...	6/33 (18%)	2/32 (6%)	0/33
C22	NH_2-ACNTIGGYECGGGS...	0/33	1/32 (3%)	2/32 (6%)
RC	NH_2-ACVWPLLSPCGGGS...	N/A	N/A	N/A

Table II. Peptide sequences of enriched phage clones.

Kinetic analysis: Kinetic analyses were performed on a resonant mirror biosensor at 25°C according to the methods described by the manufacturer (Affinity Sensors, Saxon Way, Bar Hill, Cambridge)). Briefly, Streptavidin was captured on to a biotin-coated biosensor cuvette, to which the biotinylated peptides or *Nm*LOS 3,7,9 were immobilised. The buffer used in the kinetic analysis was PBS with 0.5% (v/v) Tween-20, identical to the bio-

panning buffer. The kinetic data were analysed by curve fitting software (FASTfit v2.01, Affinity Sensors). K_{OFF} rates (arc second response per second) were determined by dilution to zero concentration of various concentrations of the mAb 9-2-L379 in the biosensor cuvette. The dissociation rate constants (K_{diss}) were then calculated by averaging the K_{OFF} rates. The association rate constants (K_{ass}) were determined from the slope of the linear regression analyses of the K_{ON} rates plotted as a function of mAb concentration. Figure 7 shows the results of kinetic analyses performed on a resonant mirror biosensor. The association rate constants (K_{ass}) were calculated from the slopes of the K_{ON} rates versus mAb 9-2-L379 concentration. The binding interactions are monitored routinely 3 times per second and the data averaged during the initial monophasic part of the binding interaction. Linear regression analysis was then used to best-fit the data. Error bars indicate standard deviations. The dissociation equilibrium constant (K_D) was calculated from K_{diss} / K_{ass}. Table III shows binding affinity constants (K_D) of the anti-*Nm*LOS 3,7,9 mAb 9-2-L379 to its native LOS3,7,9 antigen and to consensus cyclic and linear peptides identified with this mAb.

Table III

Immobilised ligand	Binding affinity (K_D) nM
*Nm*LOS3,7,9	7.5 ± 3.8
Cyclic heptapeptide - ACSWLHQPYC (C10)	11.8 ± 0.28
Linear heptapeptide (SMYGSYN)	413 ± 41
Linear heptapeptide (APARQLP)	490 ± 151

Phage particle recovery: Microtitre plate wells were coated with the anti-*Nm*LOS 3,7,9 monoclonal antibody 9-2-L379 as described above. 10^{11} phage particles were interacted with the immobilised mAb for 60 min at room temperature. Non-interacting phage were washed off with pH 7 phosphate buffered saline containing 0.5% Tween-20. Interacting phage were then removed by addition of Tris-glycine buffer pH 2.2 containing 1mg/ml BSA. The number of phage recovered were determined from transfecting *E. coli* cells with log dilutions of the phage suspension and counting the plaque forming units on an *E. coli* lawn. The results are shown in Figure 8.

Phage binding assay: Following four rounds of bio-panning with the anti-*Nm*LOS mAb 9-2-L379 phage clones expressing the cyclic heptapeptides C10, B5, C19, B11, and C22 were enriched. A cyclic peptide was picked at random (RC) from the unscreened phage library as a negative control. In the phage binding assay 10^{11} phage were interacted with either anti*Nm*LOS mAb 9-2-L379, or as negative controls, an anti-*Nm*Porin mAb specific for the P1.7 subtype, or with BSA. Both mAbs and the BSA were made up to 50µg/ml and used to coat microtitre plate wells. After reacting the phage for 60 min at room temperature (~23°C) the unbound phage were washed off with pH 7 phosphate buffered saline containing 0.5% Tween-20. Interacting phage were then removed by addition of Tris-glycine buffer pH 2.2 containing 1mg/ml BSA. The acidic phage suspensions were then neutralised with 1M Tris buffer (pH 8.8). The number of phage recovered were determined by counting the plaque forming units after transfecting *E. coli* with log dilutions of phage. Error bars indicate standard deviations (n = 3). The results are shown in Figure 9.

Discussion

In this Example we investigated the potential of cyclic peptides to act as structural mimics (mimotopes) of meningococcal LOS. In this series of experiments a phage library displaying random cyclised heptapeptides that are structurally constrained by a disulphide bridge between two cysteine residues (Fig. 6), were subjected to four-rounds of bio-panning with the anti-*Nm*LOS mAb 9-2-L379. The number of recovered phage particles observed after each round of bio-panning increased (Fig. 8). This was consistent with an enrichment of phage that interacted with the 9-2-L379 mAb. The amino acid sequences of the recovered phage clones showed a frequency of consensus peptides that was greater than the previous linear peptides identified. Up to 56% of the phage population was enriched for a single peptide sequence Ala-Cys-Ser-Tryp-Leu-His-Gln-Pro-Tyr-Cys- (C10; amino acid residues in blue are coded by the phage DNA to enable the peptide to cyclise), with several other peptides only varying from this consensus peptide by a single amino acid substitution at one or two positions only (Table II). Furthermore, the frequency of the C10 sequence was increased as the stringency of the bio-panning was raised by decreasing the concentration of the 9-2-L379 mAb in the fourth round from 50µg/ml to 0.5µg/ml (Table II). To assess the specificity of the consensus peptides a phage binding assay was performed. Phage clones expressing the consensus peptides were amplified and 10^{11} phage particles were interacted for 60 minutes with either immobilised 9-2-L379 mAb, or as negative controls with a sub-typing mAb specific for the meningococcal Porin protein antigen P1.7, or with BSA. As an additional negative control a phage clone was picked at random from the original phage library and interacted with either mAb 9-2-L379, P1.7, or BSA. The non-interacting phage particles were

removed by thorough washing in neutral pH buffer. Bound phage were then eluted off by reducing the buffer pH to 2.2 and the number of phage particles determined. Between 10^8 - 10^9 phage particles expressing the consensus peptides were bound to the 9-2-L379 mAb compared with 10^4 - 10^6 against the P1.7 mAb and BSA (Fig. 9). A phage clone expressing a random peptide also gave phage titres of around 10^5 when reacted with either the mAb 9-2-L379, the P1.7 antibody, or BSA (Fig. 8). Real-time kinetic analysis of the mAb 9-2-L379 to the C10 peptide revealed a binding affinity that is similar to the affinity to its native LOS antigen (Fig. 7 and Table III). Furthermore, reducing the disulphide bridge which constrains the cyclic peptide dramatically lowered its binding affinity to mAb 9-2-L379 (data not shown). No measurable binding was observed to the P1.7 sub-typing mAb.

In conclusion, the phage enrichment, phage binding and kinetic data suggest that structurally constrained peptides have the potential to be much better mimotopes than linear peptides.

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CLAIMS

1. A component for a vaccine against serogroup B meningococci, which comprises a mimotope of a surface lipooligosaccharide of a serogroup B meningococcus.

2. A component according to claim 1, wherein the mimotope is antigenically cross-reactive with a monoclonal antibody of high affinity to the surface lipooligosaccharide.

3. A component according to claim 1 or claim 2, wherein the mimotope comprises a peptide epitope.

4. A component according to claim 3, wherein the peptide epitope is obtainable by screening a peptide library with the monoclonal antibody of claim 2.

5. A component according to claim 3 or claim 4, wherein the peptide epitope is present in a heptapeptide.

6. A component according to claim 5, wherein the heptapeptide is selected from

IHRQGIH HIGQRHI LPARTEG GETRAPL APARQLP PLQRAPA
KQAPVHH HHVPAQK LQAPVHH HHVPAQL LPSIQLP PLQISPL
NELPHKL LKHPLEN KSPSMTL LTMSPSK AGPLMLL LLMLPGA
WSPILLD DLLIPSW LSMHPQN NQPHMSL HSMHPQN NQPHMSH
SMYGSYN NYSGYMS TNHSLYH HYLSHNT HAIYPRH HRPYIAH
TTYSRFP PFRSYTT TDSLRLL LLRLSDT SFATNIP PINTAFS

7. A component according to claim 6, wherein the heptapeptide is SMYGSYN or APARQLP.

8. A component according to claim 3 or claim 4, wherein the mimotope comprises an oligopeptide which is structurally more constrained than an unsubstituted linear form of the oligopeptide.

9. A component according to claim 8, wherein the oligopeptide comprises a cyclic peptide.

10. A component according to claim 9, wherein the cyclic peptide comprises a cyclised portion which comprises an amino acid sequence, the terminal amino acids of which are linked together by a covalent bond.

11. A component according to claim 10, wherein the cyclised portion comprises a heptapeptide.

12. A component according to claim 11, wherein the heptapeptide has the sequence SWXHXPY, wherein each X is the same or different and is an amino acid.

13. A component according to claim 12, wherein the heptapeptide has the sequence SWLHQPY.

14. A component according to claim 13, wherein the oligopeptide comprises ACSWLHQPYC.

15. A component according to any one of the preceding claims, wherein a carrier is conjugated to the mimotope to enhance the immunogenicity thereof.

16. A component according to claim 15, wherein the carrier comprises an immunogenic protein.

17. A component according to any one of the preceding claims, in which a plurality of mimotopes of the surface lipooligosaccharide is present, each mimotope being the same or different.

18. A component according to claim 17, wherein the plurality of mimotopes is conjugated together.

19. A component according to any one of the preceding claims, wherein the serogroup B meningococcus is *Neisseria meningitidis*.

20. A vaccine comprising a component according to any one of the preceding claims and an adjuvant.

21. A method for producing a component for a vaccine against serogroup B meningococci, which method comprises screening with a monoclonal antibody a mimotope library comprising candidate mimotopes, and selecting at least one candidate mimotope with high affinity to the monoclonal antibody, wherein the monoclonal antibody has high affinity to a surface lipooligosaccharide of a serogroup B meningococcus.

22. A method according to claim 21, wherein the mimotope comprises a peptide epitope.

23. A method according to claim 22, wherein the mimotope library comprises a peptide library comprising candidate peptides.

24. A method according to claim 23, wherein each candidate peptide is a heptapeptide.

25. A method according to claim 23, wherein each candidate peptide comprises a cyclic peptide.

26. A method according to any one of claims 21 to 25, which further comprises conjugating a carrier to the at least one candidate mimotope with high affinity to the monoclonal antibody to enhance the immunogenicity thereof.

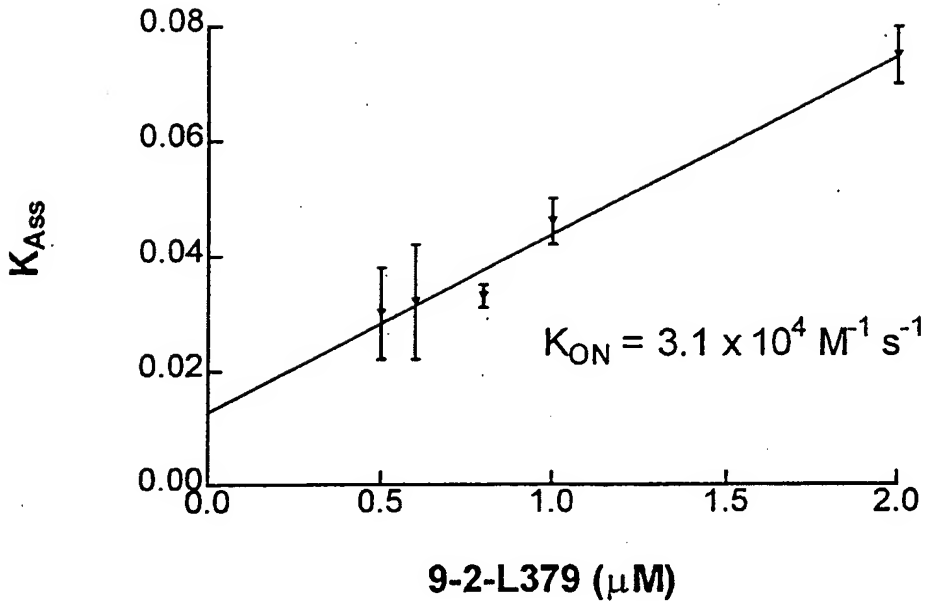


Fig. 1.

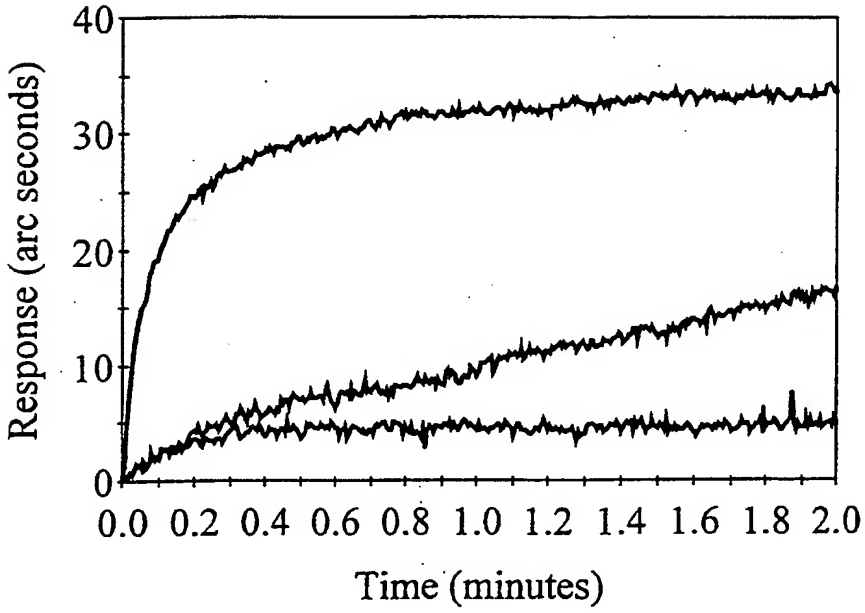
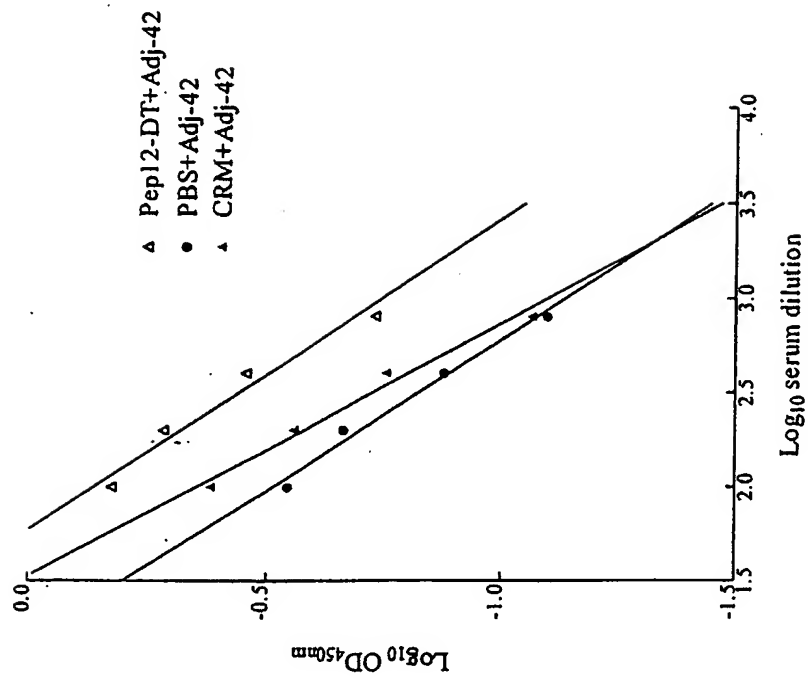


Fig. 2



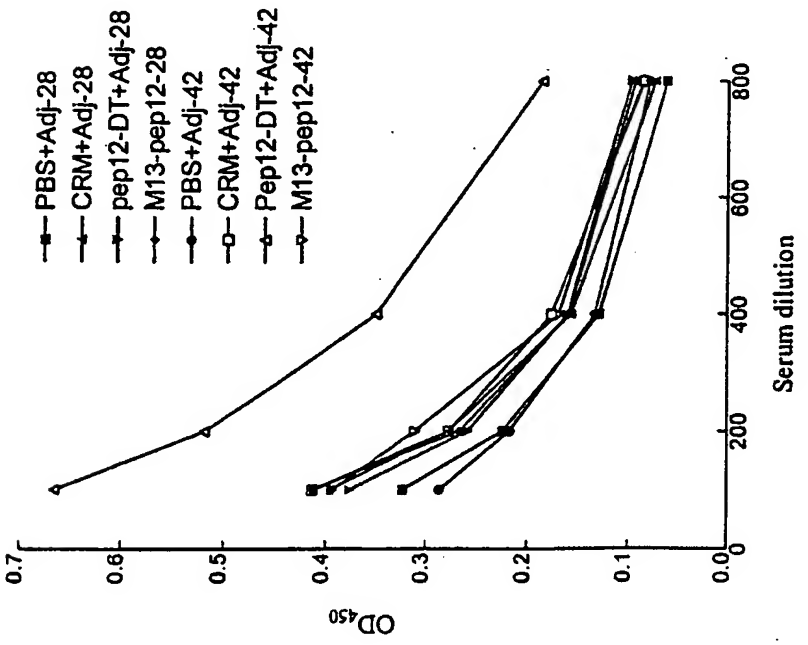


Fig. 3

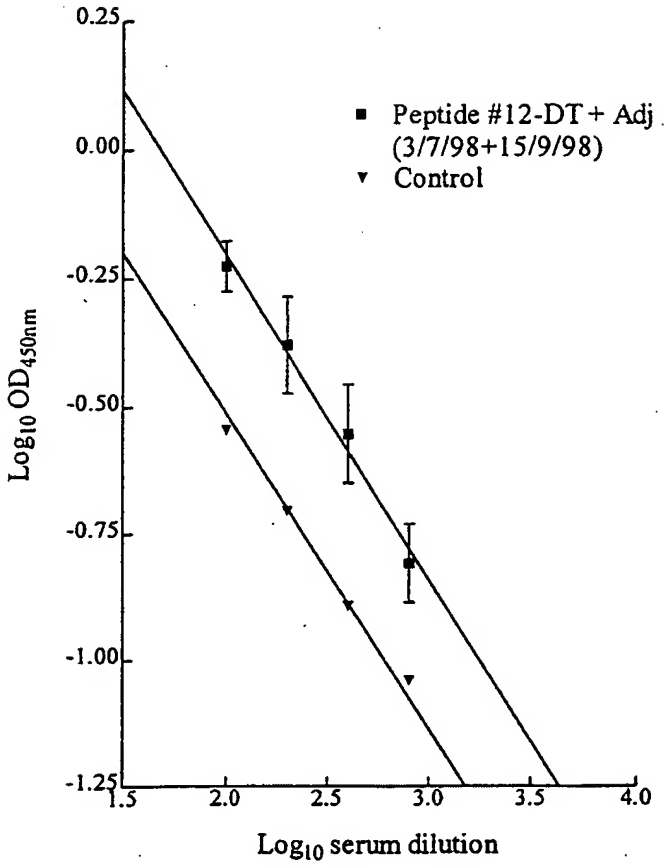


Fig. 4

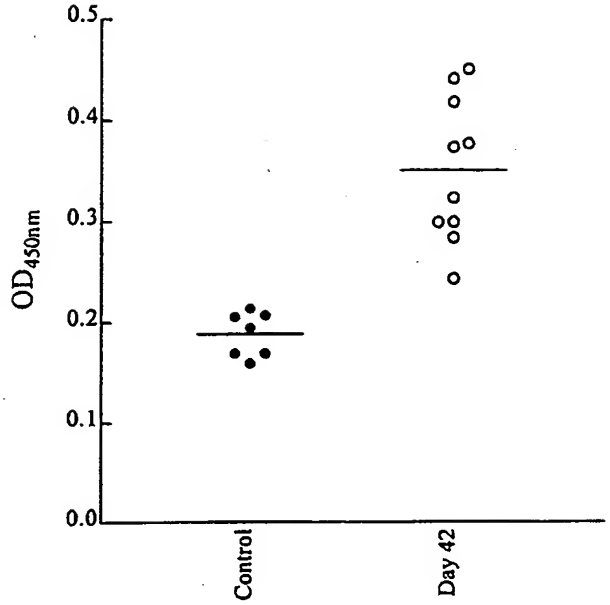
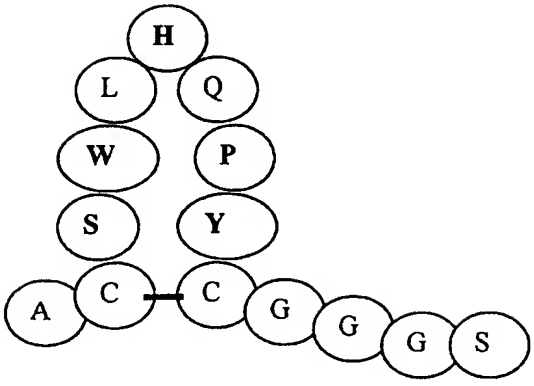


Fig. 5



Key:
A=alanine
C=cysteine
S=serine
W=tryptophan
L=leucine
H=histidine
Q=glutamine
P=proline
Y=tyrosine
G=glycine

FIGURE 6

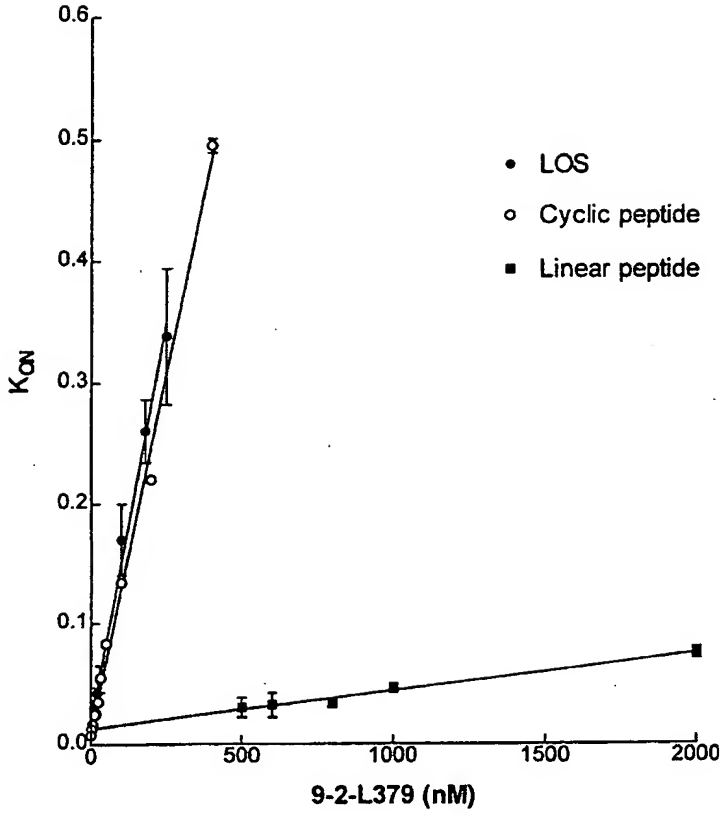

0.6
0.5
0.4
0.3
0.2
0.1
0.0
K_{ON}
0
500
1000
1500
2000
9-2-L379 (nM)
LOS
Cyclic peptide
Linear peptide

FIGURE 7

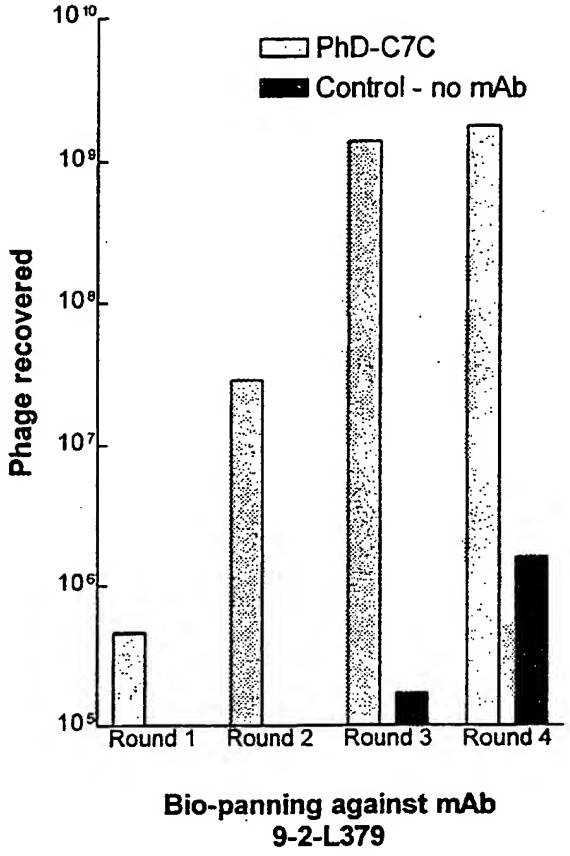

PhD-C7C
Control - no mAb
Phage recovered
10^10
10^9
10^8
10^7
10^6
10^5
Round 1
Round 2
Round 3
Round 4
Bio-panning against mAb
9-2-L379

FIGURE 8

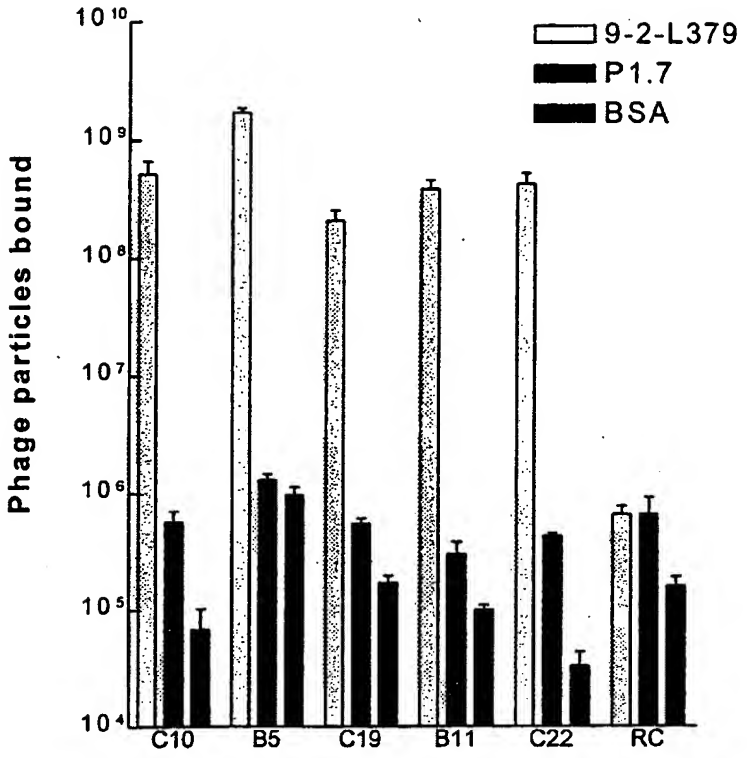

9-2-L379
P1.7
BSA
Phage particles bound
10 10
10 9
10 8
10 7
10 6
10 5
10 4
C10
B5
C19
B11
C22
RC

FIGURE 9